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Structural Insight into the Human Immunodeficiency Virus Vif SOCS Box and Its Role in Human E3 Ubiquitin Ligase Assembly▽†

Bradford J. Stanley,[1] Elana S. Ehrlich,[2] Leslie Short,[1] Yunkai Yu,[2] Zuoxiang Xiao,[2] Xiao-Fang Yu,[2]* and Yong Xiong[1]*

Department of Molecular Biophysics and Biochemistry, Yale University, P.O. Box 208114, New Haven, Connecticut 06510,[1] and Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland 21205[2]

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Human immunodeficiency virus (HIV) virion infectivity factor (Vif) causes the proteasome-mediated destruction of human antiviral protein APOBEC3G by tethering it to a cellular E3 ubiquitin ligase composed of ElonginB, ElonginC, Cullin5, and Rbx2. It has been proposed that HIV Vif hijacks the E3 ligase through two regions within its C-terminal domain: a BC box region that interacts with ElonginC and a novel zinc finger motif that interacts with Cullin5. We have determined the crystal structure of the HIV Vif BC box in complex with human ElonginB and ElonginC. This complex presents direct structural evidence of the recruitment of a human ubiquitin ligase by a viral BC box protein that mimics the conserved interactions of cellular ubiquitin ligases. We further mutated conserved hydrophobic residues in a region downstream of the Vif BC box. These mutations demonstrate that this region, the Vif Cullin box, composes a third E3-ligase recruiting site critical for interaction between Vif and Cullin5. Furthermore, our homology modeling reveals that the Vif Cullin box and zinc finger motif may be positioned adjacent to the N terminus of Cullin5 for interaction with loop regions in the first cullin repeat of Cullin5.

Virus-host interactions play critical roles in the conflict between host fitness and viral survival. Host immune systems have evolved a vast repertoire of tactics to suppress viral infections, and viruses, in turn, have developed an equally impressive arsenal of methods to evade these host defenses. A common evasion strategy used by viruses is the destruction of specific host defense proteins by hijacking the host ubiquitination-proteasome pathway (2). Viruses have evolved accessory proteins that mimic the substrate receptor molecule in host ubiquitin ligases to target cellular defense proteins for polyubiquitination, a signal for proteasome-mediated degradation. One such viral protein is the human immunodeficiency virus (HIV) virion infectivity factor (Vif) that targets the human antiviral protein APOBEC3G by mimicking cellular suppressor of cytokine signaling (SOCS) box proteins (8, 24, 33, 35, 42) (Fig. 1). In the absence of Vif, APOBEC3G causes extensive C-to-U mutations in viral reverse transcripts (12, 18, 22–24, 44) and may also physically block reverse transcription (4, 14, 29), rendering the virus ineffective.

A normal cellular elongin-cullin-SOCS-box (ECS) ubiquitin ligase is comprised of ElonginB (EloB), ElonginC (EloC), Cullin5 (Cul5), Rbx2, and a SOCS-box protein (Fig. 1). SOCS box proteins were first identified as negative regulators of cytokine signaling and are characterized by a conserved interaction domain that provides a link between cellular substrates and the E3 ubiquitin ligase. This conserved domain, the SOCS box, includes the BC box, which binds EloB and EloC (EloBC), and the Cullin box that has been proposed to participate in the binding of either Cul5 or Cul2 (16). Since its original discovery, the BC box consensus sequence has expanded to include a more diverse range of sequences, as more EloBC binding proteins have been identified (31, 41, 43). Existing crystal structures show that the SOCS box has a conserved structure composed of a BC box helix, a short 90° turn, a second short helix ending in a proline-rich region, a loop, and a third helix (Fig. 1B) (6, 34, 36). For clarity, we shall refer to the first helix as the BC box and the rest as the Cullin box.

Despite the sequence diversity among different HIV isolates, Vif uses conserved regions to orchestrate the degradation of APOBEC3G. Vif functions as the APOBEC3G substrate receptor molecule by mimicking the SOCS box component of a cellular E3 ubiquitin ligase (Fig. 1). It has been proposed that the Vif N-terminal domain interacts with APOBEC3G (8, 24), while its C-terminal half recruits the E3 ligase through two conserved regions. HIV Vif contains a BC-box region (residues 144 to 155) that interacts with EloC and is essential for recruiting the cellular E3 ubiquitin ligase (26, 42, 43). A second E3 ligase-interacting motif is the conserved H-x_5-C-x_{17-18}C-x_{3-5}-H (HCCH) zinc-binding motif that spans Vif residues 108 to 139 and interacts with Cul5 (20, 27, 30, 37, 38). Mutation of the critical His or Cys residues, or the addition of a zinc chelator, abolishes the Vif-Cul5 interaction (37).

The interaction between a third Vif C-terminal region, the Vif Cullin box, and the E3 ubiquitin ligase has not been extensively studied. Although the Vif-Cul5 interaction is confirmed by several independent experiments (17, 26, 27, 42),

* Corresponding authors. Mailing address for Y. Xiong: Department of Molecular Biophysics and Biochemistry, Yale University, P.O. Box 208114, New Haven, CT 06520-8114. Phone: (203) 436-2608. Fax: (203) 432-1296. E-mail: yong.xiong@yale.edu. Mailing address for X.-F. Yu: Department of Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe St., Baltimore, MD 21205. Phone: (410) 955-3768. Fax: (410) 614-8263. E-mail: xfyu@jhsph.edu.

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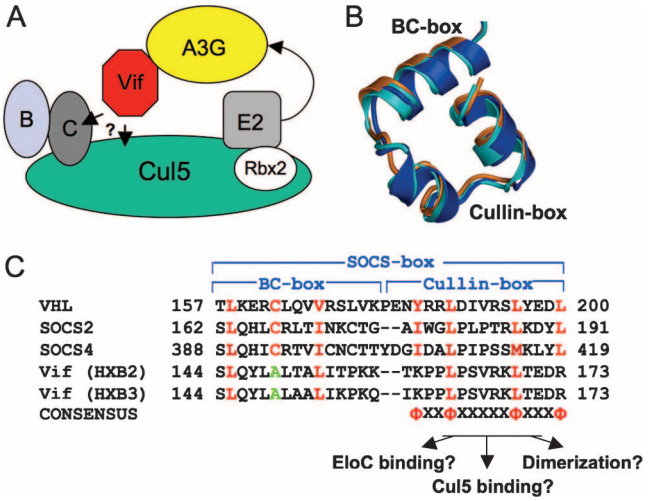

FIG. 1. Sequence and structural alignments of SOCS-box proteins. (A) Schematic depicting the E3 ubiquitin ligase complex composed of HIV Vif, EloB, EloC, Cul5, and Rbx2 that polyubiquitinates APOBEC3G. Both the HIV-1 HXB2 (GenBank accession no. K03455) and the HXB3 (GenBank accession no. EU541617) sequences are shown as the Vif crystal structure was obtained with the HXB3 sequence, but mutational work was performed with the HXB2 sequence. (B) Superimposition of the SOCS boxes of SOCS2 (blue), SOCS4 (cyan), and VHL (orange) shows a highly conserved structure despite different amino acid sequences. (C) Alignment of the Vif SOCS box sequence with cellular SOCS-box protein sequences shows a series of conserved hydrophobic amino acids (shown in red). The alanine in Vif that replaces the canonical cysteine in other SOCS box proteins is colored green.

there has been no evidence of a direct interaction between the Vif Cullin box and Cul5. Cullin boxes have been shown to mediate interaction with Cul5 or Cul2 in a number of cellular SOCS-box proteins (16); however, the sequence of the Vif Cullin box only partially matches the previously proposed Cul5 box consensus (16) (Fig. 1C). In addition, a proline-rich region in the Vif Cullin box (161-PPLP-164) has been previously implicated in Vif homo-oligomerization and shown to be important to Vif function (39, 40), but its exact functional relevance has not been established.

Despite the rapid accumulation of biochemical and genetic data, the details of how HIV Vif recruits the E3 ubiquitin ligase largely remain unknown because of the lack of any structural data on Vif. We conducted a series of experiments to obtain structural insight into the interactions between the Vif SOCS box and the E3 ligase. We have obtained a crystal structure of the HIV Vif BC-box in complex with human EloBC. This provides structural evidence that a viral BC-box hijacks a host E3 ligase by mimicking the conserved binding interface used by cellular SOCS-box proteins. We further performed a mutational analysis to demonstrate direct interaction between the Vif Cullin box and Cul5. The structural and mutational data, together with available structures of Cullin complexes, allowed us to propose a homology model of the complex between the Vif SOCS box, EloBC, and Cul5. This model provides further insight into how the HIV Vif viral SOCS box assembles with a cellular ubiquitin ligase to polyubiquitinate APOBEC3G.

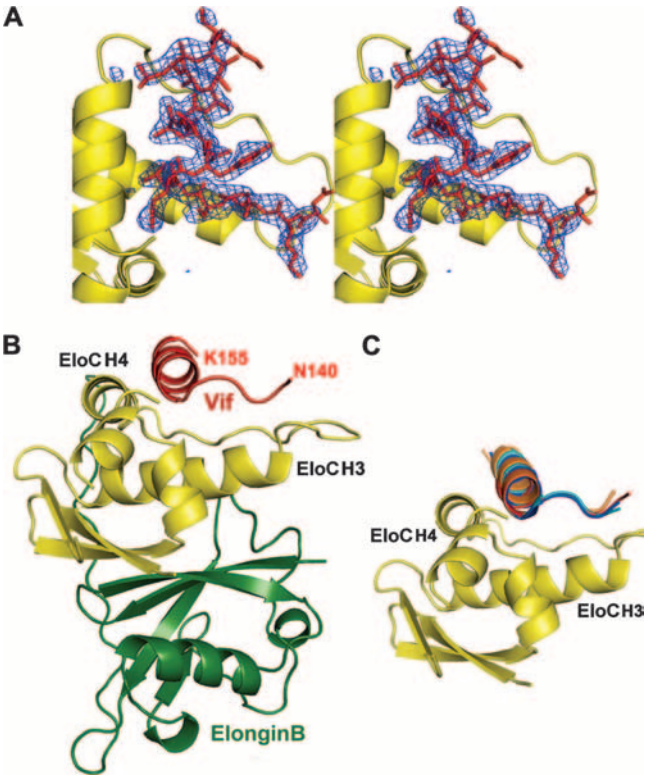

FIG. 2. Structure of the HIV Vif BC box in complex with EloB and EloC. (A) Stereo view of a simulated annealing omit map of Vif BC-box electron density (3σ level) calculated using CNS. A stick representation of the final model is shown in the map. (B) The overall structure of the Vif BC box (red), EloC (yellow), and EloB (green). (C) The Vif BC box (red) binds to EloC similarly to the cellular SOCS box proteins VHL (orange), SOCS2 (blue), and SOCS4 (cyan).

MATERIALS AND METHODS

Cloning, expression, and purification of Vif/EloB/EloC complex. HIV Vif HXB3 amino acids 139 to 176 were cloned into pETDuet-1 with an N-terminal His_6 tag and TEV protease cleavage site. This plasmid was cotransformed into BL21(DE3) cells with pACYCDuet-1 containing EloB and EloC (6). The Vif-EloB-EloC complex was purified on a nickel affinity column, and fractions containing the Vif-EloB-EloC complex were pooled, concentrated, and incubated at 4°C overnight with TEV protease. A second nickel affinity step was used to remove the TEV and cut tag, and then the flowthrough was concentrated and loaded onto a Superdex-75 gel filtration column. Fractions containing the Vif-EloB-EloC complex were pooled and concentrated to approximately 7 mg/ml in 25 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 1 mM dithiothreitol.

Crystallization and structure determination. Crystals were obtained by hanging-drop vapor diffusion in 0.1 M Tris-HCl (pH 7.0) and 40% PEG 350 monomethyl ether. Crystals were frozen in liquid propane with the reservoir solution. Due to the high polyethylene glycol concentration, the reservoir solution made a suitable cryoprotectant. Diffraction data were collected at the Advanced Photon Source NECAT beamline ID24 (Argonne, IL) and the Brookhaven National Laboratory beamline X29 (Upton, NY). The crystals belonged to space group $P2_12_12_1$ and diffracted to 2.4 Å. The structure was resolved by molecular replacement using the coordinates of EloB/EloC from the crystal structure of EloB/EloC/VHL (PDB ID 1vcb) (34). Two copies of the EloB/EloC complex were found in the asymmetric unit of the of the crystal with the program Phaser (25). After rigid body and restrained refinement in Refmac (28), the Vif BC-box region, which was not include in the model, was clearly visible in the 2Fo-Fc electron density map. The electron density was further improved by twofold multidomain noncrystallographic symmetry (NCS) averaging using the program DM (9). Two NCS-related copies of the Vif BC box (residues 140 to 155) were then built into the electron density using the programs O (15) and Coot (11). A simulated annealing omit map with the Vif BC-box omitted (Fig. 2A) was

TABLE 1. Crystal data and refinement statistics

Parameter	Value[a]
Space group	$P2_12_12_1$
Unit cell a, b, c (Å)	55.51, 66.91, 122.64
Resolution (Å) (range)	30–24 (2.49–2.4)
R_{merge} (%)	9.6 (44.1)
I/σI	11.7 (2.5)
% Completeness	98.2 (97.3)
Redundancy	3.9 (3.3)
R/R-free (%)	18.6/23.5 (18.9/26.9)
Ramachandran angle	
% Preferred regions	94.4
% Allowed regions	5.0
% Outliers	0.6
RMSD, bond (Å)	0.008
RMSD, angle (°)	1.1

[a] Values in parenthesis are for the highest-resolution shells.

calculated in CNS (5) to verify the model built. Iterative cycles of model building and refinement were subsequently carried out by using Refmac (28). Strong NCS restraints were imposed throughout refinement since relaxing them caused an increase in the R-free factor. The final model had good geometry, with an R/R-free factor of 18.6/23.5%. The data and refinement statistics are summarized in Table 1.

Homology modeling of Vif-EloB-EloC-Cul5 complex. The Cullin-box region of Vif was homology modeled based on the crystal structure of SOCS2 (6). The Cullin box of SOCS2 (amino acids 158 to 193) was grafted onto the Vif BC-box/EloBC crystal structure by superimposing the BC-box regions of the two structures in Coot (11). The Vif Cullin box was modeled by mutating the SOCS2 Cullin-box sequence to the Vif Cullin-box sequence in Coot and the geometry was idealized in Refmac (28). The highly conserved structure of cellular SOCS boxes (Fig. 1B) imparts confidence in the homology modeling of the Vif Cullin box.

The Vif SOCS-box/EloBC homology model from above was used to examine its interaction with Cul5. The crystal structure of Cul1 from the Skp2-Skp1-Cul1-Rbx1 (SCF) ternary complex (45) was used as a template for homology modeling of Cul5. Two homology modeling programs, Modeler (32) and 3D-JIGSAW (3), produced similar results. Prior to obtaining a Cul5 homology model from Modeler, multiple sequence alignment was carried out with Cul1, Cul2, Cul4A, and Cul5 by using the program MUSCLE (10) (see Fig. S2 in the supplemental material). The availability of two crystal structures of different Cullins, Cul1 (45) and Cul4A (1), helped to validate and manually improve the alignment. The aligned sequences of Cul5 and Cul1 were input into Modeler for homology modeling. Alternatively, the automated homology modeling program 3D-JIGSAW generated a similar model with flexible loop regions in different conformations. These loop regions, discussed in Results below, are likely to form contact points between Vif and Cul5. The Vif SOCS-box/EloBC homology model was subsequently docked onto the modeled Cul5 by superimposing EloC and Cul5 onto Skp1 and Cul1, respectively, in the SCF crystal structure (see Fig. S1 in the supplemental material). The program Coot was used for the superpositioning. The geometry of the final Vif SOCS-box/EloBC/Cul5 complex model was idealized in Refmac.

Plasmids and antibodies. The following HIV-1 Vif expression vectors have been described previously: wild-type Vif-hemagglutinin (HA), wild-type Vif-myc, L145A, L163S, ΔSLQ, L169S, and SIV Vif (20, 42, 43). Vif S144A, L148A, L153A, Q146A, and AALA were constructed by changing S144, L148, L153, Q146, and the prolines at positions 160 to 163 to A, respectively, using site-directed mutagenesis. The following antibodies have been described previously: HA, myc, Cul5, ElonB, and EloC (20, 42, 43).

Cells, transfection, and immunoprecipitation. 293T cells were maintained as suggested by the American Type Culture Collection. Transfections were carried out by using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. At 48 h posttransfection cells were harvested for immunoprecipitation as described previously (20, 42, 43). Immunoprecipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by immunoblotting against the indicated proteins.

Data deposition. Coordinates and structure factors have been deposited in the Protein Data Bank under accession code 3DCG.

RESULTS

Crystal structure of the HIV Vif BC box in complex with EloBC. We have determined the crystal structure of the Vif BC box-EloBC complex at 2.4-Å resolution (Fig. 2A). The complex structure was solved by molecular replacement using the coordinates of EloBC from the VHL-EloBC complex (PDB ID 1vcb) (34). Clear electron density was observed in the 2Fo-Fc map for Vif residues 140 to 155 before their inclusion in the model. These residues correspond to the Vif BC-box helix that includes the consensus sequence of SLQYLA. The C terminus of the Vif construct, containing the Cullin box, was disordered and not observed in the crystal structure. Mass spectrometry of dissolved crystals confirmed that Vif had not been degraded (data not shown).

The Vif BC box adopts a loop-helix structure, with the helix starting at the second residue (L145) in the consensus SLQYLA sequence (Fig. 2B). Both the loop and the helix form a largely hydrophobic interface that binds EloC. Extensive hydrophobic interactions occur between side chains of the Vif BC box and those of helix 3 and helix 4 of EloC. Vif residues V142, L145, L148, A149, A152, and L153 create the hydrophobic face that interacts with EloC (Fig. 3A and B). Hydrogen bonding between the backbone carbonyl of Vif G143 and EloC Y76 also contributes to the Vif-EloC interaction. Similar interactions are made by cellular BC box-EloBC complexes (6,

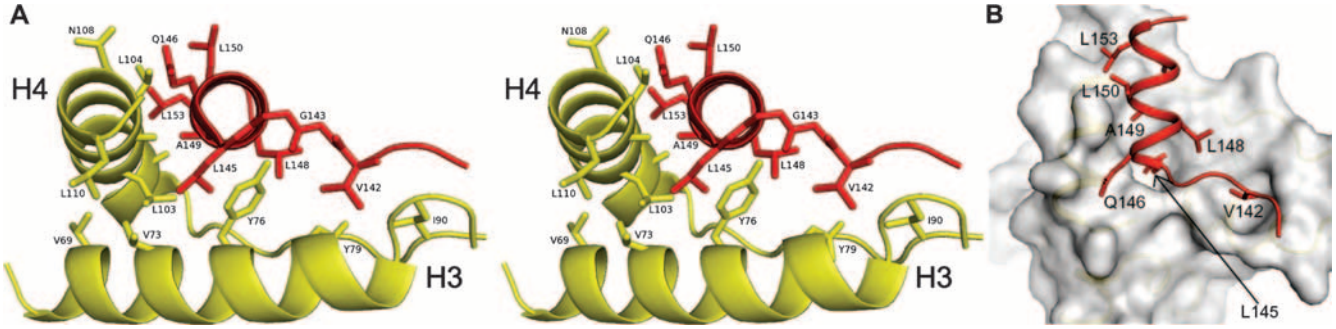

FIG. 3. Detailed interactions between HIV Vif and EloC. (A) Stereo view of the HIV Vif BC box (red) shows the extensive hydrophobic interactions it makes with EloC (yellow) helices 3 and 4. For clarity, only helices 3 and 4 of EloC are drawn. (B) A transparent surface representation shows binding pockets for Vif V142 and L145 on the surface of EloC. The secondary structure of EloC is shown in yellow under the transparent surface, and the Vif BC box is shown in red.

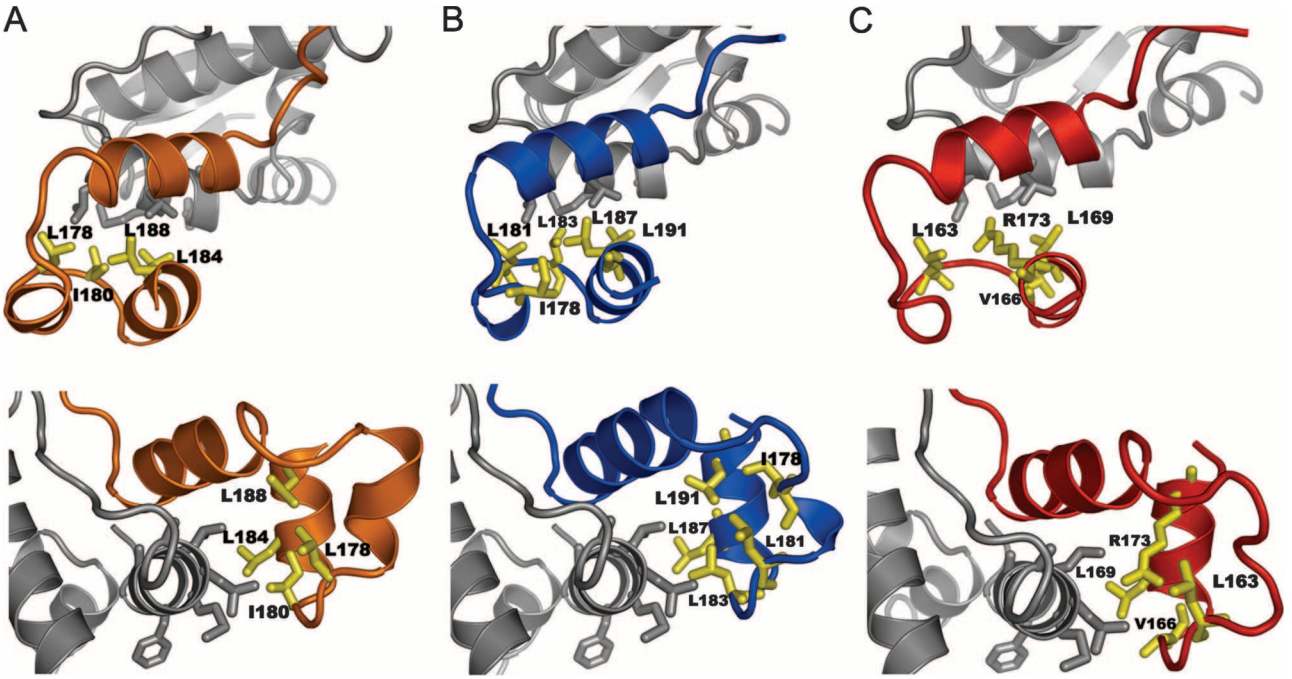

FIG. 4. SOCS box proteins have a conserved Cullin-box structure that is anchored to EloC by conserved hydrophobic residues. (A) Conserved hydrophobic residues (yellow) of VHL anchor the Cullin box to EloC (gray). Lower panel shows the interface by viewing down EloC helix 4. (B) SOCS2 makes similar hydrophobic contacts with EloC and preserves the same structure as VHL. (C) Homology model of the Vif Cullin box shows hydrophobic interactions with EloC similar to that of VHL and SOCS2.

7, 34, 36), which adopt a conformation like that of the viral Vif BC box (Fig. 2C). A notable difference between the cellular and viral BC boxes is that the highly conserved cysteine and arginine residues in the middle of the cellular BC box are replaced by alanine (A149) and leucine (L150) in Vif (19, 41) (Fig. 1C). The alanine residue in Vif is located at a position that has the closest Cα-Cα distance between the BC-box helix and helix 4 of EloC. The position of the Vif BC box relative to EloC is the same as those in the SOCS2 (6) and SOCS4 (7) structures but slightly shifted from that in the VHL (34) structure by about 1 Å at its C terminus. The two copies of the Vif-EloB/EloC complex in the asymmetric unit are identical when superimposed.

The Vif Cullin box is predicted to form a second hydrophobic interface with EloC. To delineate the role of the Vif Cullin box, we first analyzed the known structures of cellular SOCS box proteins. Although the Cullin-box amino acid sequences of all SOCS box proteins solved to date are different (Fig. 1C), their structures remain essentially the same (Fig. 1B). For example, the SOCS box sequences of SOCS2 (amino acids 161 to 193) and VHL (amino acids 156 to 190) are only 18% identical. However, superimposition of their structures using the program DALI (13) gives a root mean square deviation (RMSD) of 0.7 Å between their backbones and a Z-score of 5.9 (a Z-score higher than 3 is considered significant). Even with a two-amino-acid insertion in VHL (P172-E173), the rest of its Cullin box aligns with SOCS2 nearly identically (Fig. 1B).

Comparison of existing Cullin box structures reveals that the conserved hydrophobic residues in this region (Fig. 1C), mostly leucines, point toward a different face of the same EloC helix (H4) that interacts with the BC box (Fig. 4A and B). Using SOCS2 as an example, I178, L181, L183, L187, and L191 all face inward toward a hydrophobic pocket formed at the EloC–BC-box interface (6) (Fig. 4B). Similarly, L178, I180, L184, and L188 of VHL interact with this same EloC–BC-box interface (Fig. 4A) despite the fact that its sequence does not follow the Cullin box consensus (Fig. 1C). Overall, Vif/VHL share six identical residues in their last 11 Cullin-box residues, including leucine 163/178, valine 166/181, arginine 167/182, leucine 169/184, glutamate 171/186, and aspartate 172/187 (Fig. 1C). This is a higher degree of sequence homology than is observed between the VHL and SOCS2 Cullin boxes, which superimpose almost identically. Vif's high level of sequence identity to VHL in the Cullin box motif, which has a highly conserved structure, suggests that the Vif Cullin box may form the same structure. This allowed us to carry out homology modeling of the Vif Cullin box by directly grafting the Vif sequence onto the SOCS2 Cullin-box structure. In this model, the conserved Vif leucines (L163 and L169) make hydrophobic interactions that position the Vif proline-rich loop and the rest of the Cullin box along helix 4 of EloC (Fig. 4C).

Although the Cullin box in HIV-1 Vif is apparent, HIV-2 and SIV Vif do not have readily distinguishable Cullin boxes. It is still possible that the conserved structure of the Cullin box does form in HIV-2 and SIV Vif even though there may be limited sequence similarity. Also, a recent study of Cullin-box sequences (21) has shown that the spacing between the BC box and the Cullin box is quite variable. Considering the divergence between the Cullin box from HIV Vif and SOCS2/SOCS4/VHL, it is possible that the HIV-2 and SIV Cullin boxes have diverged even further and/or are not necessarily adjacent to the BC box. Alternatively, HIV-2 and SIV Vif may

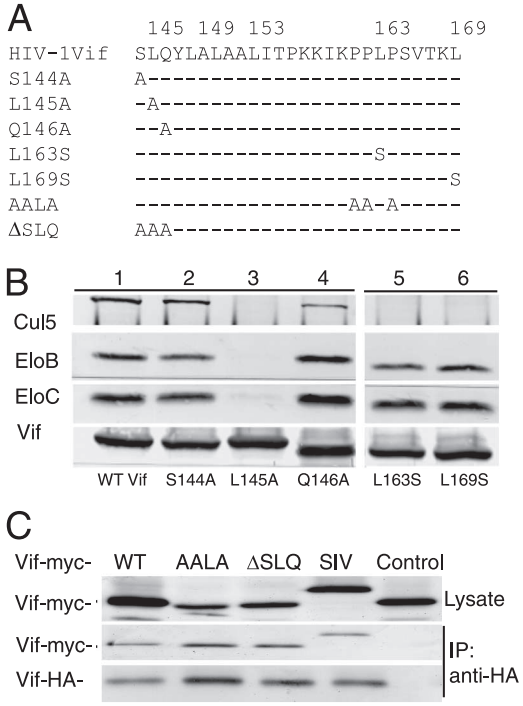

FIG. 5. Mutation of conserved residues in the Vif SOCS box. (A) Map of alanine substitutions in the Vif BC box used in immunoprecipitation experiments. (B) Anti-Vif immunoprecipitations were Western blotted with antibodies against EloB, EloC, Cul5, and Vif. EloB and EloC binding is affected by mutation of critical BC-box residues, and Cul5 binding is lost upon mutation of downstream conserved hydrophobic residues. (C) Vif oligomerization is independent of conserved residues in the Vif SOCS box. Cells were cotransfected with myc-tagged wild-type or mutant Vif expression vectors and HA-tagged wild-type Vif expression vectors. HA-tagged proteins were immunoprecipitated with anti-HA conjugated agarose and Western blotted with antibodies against HA- and myc-tagged Vif proteins.

have evolved a different, yet functionally equivalent, motif that serves the same purpose as the Cullin box.

The Vif Cullin box-EloC interaction is required for the binding of Cul5 but not EloBC. The formation of a second interface with EloC by the Vif Cullin box raises a question: is this interaction required for Vif to bind EloBC or to position the conserved Cullin-box structure for interaction with Cul5? A series of mutations in the SOCS box of Vif were made to address this question (Fig. 5A). 293T cells were transfected with wild-type or mutant Vif expression vectors. The HA-tagged Vif proteins were then coimmunoprecipitated with endogenous Cul5, EloB, and EloC and subsequently examined by immunoblotting. In agreement with previous studies (26, 43), the L145A mutation results in loss of binding to EloB, EloC, and Cul5 (Fig. 5B, lane 3). On the other hand, mutations of the highly conserved L163 or L169 had little effect on Vif interaction with EloB and EloC (Fig. 5B, lanes 5 and 6), and interaction with Cul5 was severely inhibited compared to parental Vif. This suggests that the interaction involving the conserved leucines in the Vif Cullin box is critical for Cul5 binding, not EloBC binding.

The conserved amino acids in Vif SOCS box are not required for Vif oligomerization. To assess whether the Vif SOCS-box consensus residues play a role in Vif oligomerization as suggested by previous studies (40), we cotransfected 293T cells with HA-tagged wild-type Vif, along with a series of myc-tagged Vif expression vectors. The myc-tagged Vif expression vectors (Fig. 5A) included wild-type Vif, Vif-AALA (all prolines of the PPLP motif mutated to alanine), Vif-ΔSLQ (the SLQ consensus mutated to AAA), simian immunodeficiency virus Vif (which naturally has no PPLP yet still interacts with Cul5), and control parental vector. Our data show that Vif SOCS-box consensus mutants still interact with wild-type HIV-1 Vif (Fig. 5C), suggesting that other regions of Vif may mediate oligomerization.

Potential structural determinants for Cul5 binding. The crystal structure of the Vif BC box in complex with EloBC and our mutational evidence for the role of the Vif Cullin box in the recruitment of Cul5 enabled us to propose a homology model of Vif-Cul5 binding (Fig. 6). The crystal structure of the Cul1-Rbx1-Skp1-F box SCF ubiquitin ligase (45) was used as a template. Cul5 was modeled onto Cul1 and our Vif SOCS-box EloBC model was then positioned in the SCF complex by aligning EloC with Skp1. This SCF ubiquitin ligase structure (45) serves as a good template for modeling the Vif-EloBC-Cul5 complex for the following reasons. First, as demonstrated by the resemblance of the Cul1 (45) and Cul4A (1) structures (see Fig. S2 in the supplemental material), the cullins, being close paralogues, are likely to have very similar structures. Second, EloC is the Skp1 equivalent for Cul5-Rbx2 ubiquitin ligases, and superimposition of EloC onto Skp1 in DALI (13) results in the alignment of 82 residues with a sequence identity of 34% and a Z-score of 11.2. Lastly, structural superimposition of Skp1 and EloC has also been used by others to investigate potential interfaces between cellular SOCS box proteins and their respective cullins (6, 16).

The homology model of the Vif-EloBC-Cul5 complex reveals two potential Vif-Cul5 interfaces. The first interface is observed between the PPLP downstream region in the Vif Cullin box and Cul5 loop 2 (residues 51 to 60) (Fig. 6). Interaction at this interface is supported by our mutational data and provides an explanation for the loss of Cul5 binding upon mutation of L163 in the PPLP. The second potential interface is found between the Vif HCCH zinc finger motif and Cul5 loop 5 (residues 118 to 134) (Fig. 6). The last histidine (H139) of the HCCH motif is immediately next to the first amino acid (N140) that we see density for in our crystal structure; therefore, we can estimate the position of the zinc finger with respect to Cul5. The position of H139 indicates that this end of the HCCH motif is more than 20 Å away from any Cul5 residues except for those in loop 5. Loop 5 is variable in length and composition between the different Cullins (see Fig. S2 in the supplemental material) and could provide potential specificity determinants. In addition, loop 5 is likely to be flexible and, depending on its orientation, it could also interact with the last helix in the Vif Cullin box (Fig. 6).

DISCUSSION

HIV Vif makes a number of critical interactions with human ubiquitin ligase components EloB, EloC, and Cul5 to tether the ligase to antiviral APOBEC3G. Assembly of this ubiquitin ligase leads to the polyubiquitination and subsequent protea-

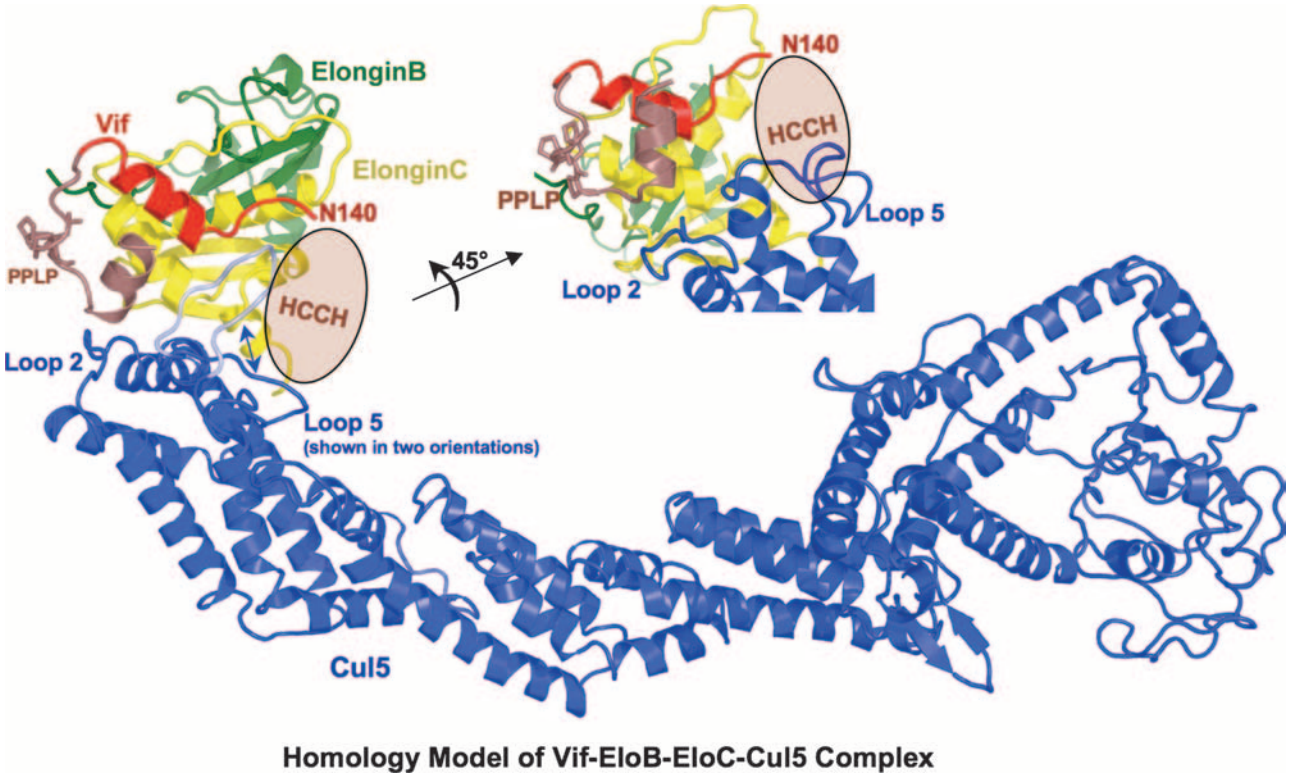

FIG. 6. HIV Vif-Cul5 interaction model. Cul5 loops 2 and 5 make potential additional interactions between Vif and Cul5. The Vif BC-box (red), EloB (green), and EloC (yellow) are from the current crystal structure; the Vif Cullin-box (brown) and Cul5 (blue) are homology modeled. Loop 5 from Cul5 is shown in two possible orientations: extended upward toward the Vif zinc finger (light blue) and folded back onto Cul5 (dark blue).

some-dependent destruction of APOBEC3G. Therefore, Vif and the interactions that it makes with the host ubiquitin ligase are of great interest because of their potential as therapeutic targets. However, our understanding of these interactions has been impaired by the lack of structural information. In order to further our understanding of how Vif interacts with components of human E3 ubiquitin ligase, we determined the structure of a Vif SOCS box construct with elongins B and C, used mutational analysis to determine the role of a downstream region that was disordered in our structure, and then proposed a model for the Vif-EloBC-Cul5 interactions.

Our current crystal structure of the Vif BC box-EloBC complex provides direct evidence for the proposed viral hijacking of human E3 ubiquitin ligase components. The viral Vif BC-box conformation and the binding interactions are similar to those observed in structures of cellular SOCS box protein-EloBC complexes (6, 7, 34, 36) (Fig. 2C). This demonstrates that HIV Vif uses a mechanism similar to that of endogenous substrate receptors to recruit the ligase for polyubiquitination of APOBEC3G. The interface between the Vif BC box and EloC is dominated by hydrophobic interactions as in cellular BC boxes, but there are some notable differences. The highly conserved cysteine in cellular BC boxes is replaced by alanine (A149) in Vif. The space between the BC box and EloC helix 4 appears to be able to accommodate an amino acid as large as a cysteine but may exclude any residue larger. This is consistent with the mutational studies at this position (43). In addition, the conserved arginine that follows the cellular BC box cysteine is a leucine (L150) in the Vif sequence. This residue is located opposite the hydrophobic face of Vif that interacts with EloC, so it is unlikely to affect the Vif-EloC interaction.

The high degree of structural conservation of all available SOCS-box structures led us to take a closer look at the roles of the conserved Cullin box residues. Superimposition of existing Cullin box structures demonstrates that the conserved hydrophobic residues in this region, mostly leucines, are involved in binding EloC (Fig. 3). Because the Vif sequence does not follow the Cullin-box consensus closely, two different sequence alignments have been reported (26, 43). Our structure-based alignment of the cellular Cullin-box sequences supports that L163 and L169 of Vif should be aligned to the conserved leucines (43) to preserve the hydrophobic interactions with EloC. By inserting the same two-residue gap in Vif as shown between SOCS2 and VHL (Fig. 1C), the Vif and VHL Cullin boxes can be aligned beginning at Vif L163 and VHL L178. This positions Vif L169 and VHL L184 as the third conserved hydrophobic residue in the consensus (Fig. 1C). Taken together, we predict that Vif L163 and L169 contribute to a second hydrophobic interface between the Vif Cullin box and helix 4 of EloC.

Our mutational data also suggest that there are two interfaces at which HIV Vif binds to EloC. Mutation of critical residues within the SOCS box indicates that the conserved residues of the BC box and Cullin box have distinct roles.

Mutation of L145 in the SOCS box abolishes EloBC and Cul5 binding (Fig. 5B). The L145 mutation contributes substantially to the hydrophobic interface between Vif and EloC by fitting into a hydrophobic pocket formed by EloC helices 3 and 4 (Fig. 3B), and therefore the L145A mutant no longer binds EloC. This hydrophobic pocket is lined by EloC residues L110, L102, V73, and V69 (Fig. 3B). Vif Cullin box mutants L163S and L169S lose the ability to bind Cul5 but retain the ability to bind EloBC. A possible explanation for these data is that the EloC-L163/L169 interactions position the Cullin box for interaction with Cul5. This would explain why the Cullin box is not required for binding to EloBC but is required for the recruitment of Cul5, as shown in a previous study in which truncated HIV Vif (residues 90 to 160) still bound EloC but not Cul5 (26). This also explains the loss of Cul5 binding to Vif when the interaction with EloBC is abolished by the L145A mutation. When binding to EloBC is lost, as in L145A, the Cullin box is not positioned properly for interaction with Cul5.

For the two Vif SOCS-box–EloC interfaces we have described, the most extensive interaction occurs between the Vif BC box and EloC. This serves as the primary attachment between Vif and the EloBC component of the ligase. The role of a secondary, less-extensive interaction between the Cullin box and EloC is not for EloBC binding, but rather for positioning the Cullin box, which is critical to binding Cul5. This second EloC-interacting interface is probably weaker than that formed by the BC box, since the distance between helices at the interface is further and the interactions are less extensive. The relatively weak interaction may explain why the Vif Cullin box region is disordered in our crystal structure. It is apparent that the crystallographic symmetry mates occupy part of the expected Cullin-box position. This also could have prevented formation of an ordered Cullin box.

Recent experimental evidence has shown that the Vif HCCH motif interacts with Cul5 (20, 27, 30, 37, 38). Our crystal structure and homology model reveals Cul5 loop 5 as a potential interaction site for the Vif HCCH motif. In our crystal structure, the observed protein density begins at Vif residue N140, which is next to the last histidine (H139) in the HCCH motif. This places the zinc finger adjacent to the N140 observed in our structure, which faces Cul5 loop 5 in our homology model (Fig. 5). The importance of loop 5 in Vif-Cul5 binding has been demonstrated in a series of deletion experiments where only the deletion of loop 5 (amino acids 119 to 139) abolished the Vif-Cul5 interaction without affecting EloBC-Cul5 binding (38). Our model, in which the Vif HCCH motif interacts with Cul5 loop 5 (Fig. 6), provides an explanation that is consistent with the deletion studies. This also is supported by the fact that Vif selectively binds Cul5 over Cul2 even though other EloBC containing ligases such as VHL bind Cul2 (34). The lengths and sequences of loop regions in Cul5 and Cul2 have notable differences and could account for this selectivity (see Fig. S2A in the supplemental material).

In the present study, we investigated HIV Vif recruitment of the cellular E3 ubiquitin ligase complex by using crystallography, mutagenesis, and homology modeling. Our crystal structure of the Vif-EloBC complex demonstrates binding of a viral BC box to a cellular ubiquitin ligase; our mutational studies reveal that the Vif Cullin box is critical to Vif-Cul5 interaction, and our homology modeling suggests potential interfaces between Vif and Cul5. These results give structural insight and may provide directions for further experiments to probe the assembly to Vif-E3 ubiquitin ligase complex.

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